Hypothalamic neurons defined by the expression of RIPCre transgene have been suggested to control body weight. However, which RIPCre neurons regulate energy expenditure, and via release of which neurotransmitter, is presently unknown. This study revealed that acute activation of arcuate RIPCre neurons innervated NTS/DMV-projecting neurons in the paraventricular hypothalamus. Thus, as additional neurons to those expressing POMC or AgRP, RIPCre neurons in the arcuate play important roles in regulating energy expenditure via releasing GABA. To assess the mechanism and function of RIPCre neurons, animals with disrupted neurotransmitter release (GABA or glutamate) from these neurons were generated by crossing RIPCre transgenic mice with lox-vGAT(vesicular GABA transporter) or lox-vGlut2 (vesicular glutamate transporter) mice. Combined with patch-clamp recordings, and channelrhodopsin2-assisted circuits mapping (CRACM), this study has explored the neural circuitry involving ARC-RIPCre neurons in regulating energy expenditure. On the other hand, the Cre-dependent halorhodopsin (eNpHR 3.0) expression has been tested following AAV-injection into the arcuate of Agrp-ires-Cre mice. In brain slice preparations, it has been confirmed that yellow light repeatedly and reversibly hyperpolarizes and silences AgRP neurons expressing NpHR. Our study suggests that the ChR2/Halo system constitutes a powerful toolbox enabling photostimulation and photoinhibition in genetically-specified neurons, enabling the systematic analysis of neural circuits.

Cardiac Electrophysiology III

3408-Pos Board B269

The Relationship Between Conduction Velocity and Atrial Arrhythmogenicity under Conditions of Altered Ca2+ Homeostasis in RyR2-P2328S Murine Hearts

James Harmsworth King, Yanmin Zhang, Chris Huang, James Fraser.
University of Cambridge, Cambridge, United Kingdom.

Atrial fibrillation (AF) is a significant contributor to mortality and health care expenditure; it is associated with abnormal sarcoplasmic reticulum (SR) Ca2+ release through cardiac ryanodine receptors. The initiation of AF requires an ectopic action potential (AP) triggered within a substrate of atria conducive to the formation of re-entry circuits. The present experiments explored whether increased diastolic SR Ca2+ release might contribute to atrial arrhythmogenicity in homozygotic gain-of-function RyR2-P2328S mice (RyR2s/s).

Electrocardiographs and intracellular APs were recorded from Langendorff-perfused hearts at regular 8 Hz (S1) stimulation. RyR2s/s hearts showed depolarised resting membrane potentials that were associated with an increased incidence of ectopic APs and sustained tachyarrhythmias. During regular stimulation, the RyR2s/s showed reduced epicardial conduction velocities (CV), measured using a 64-electrode array, that corresponded with reduced S1 AP upstroke velocities ((dV/dt)max).

When ectopic (S2) APs were imposed during S1 stimulation at progressively shorter S1S2 intervals, the inter-atrial CV and dV/dt(max) of the S2 APs decreased with each successive interval. Moderate reductions in S1S2 interval provoked sustained tachyarrhythmias in RyR2s/s hearts, whereas significantly shorter S1S2 intervals were required to provoke arrhythmias in WT hearts. Interestingly, the mean S1S2 interval required to provoke an arrhythmia in each variant resulted in similar inter-atrial CVs and dV/dt(max).

These findings suggest that triggered activity is likely to lead to a sustained tachyarrhythmia in murine atria only when the CV of an ectopic AP is slow (<~1.1 m/s). We have shown this is more likely to occur in RyR2s/s atria where increased diastolic SR Ca2+ release is associated with both increased ectopic APs and an intrinsically slow CV. We therefore suggest that abnormal Ca2+ homeostasis contributes to both the trigger and the re-entry substrate in sustained atrial tachyarrhythmias.

3409-Pos Board B270

Flash Photolysis of Caged Compounds during Simultaneous Imaging of Calcium and Voltage in the Whole Heart using Light-Emitting-Diodes

Peter Lee[1,2], Leslie M. Loew[3], Derek A. Terrar[4], Paul Ewart[2], Peter Kohl[5], **Christian Bollensdorff**[5].
[1]Life Science Interface Doctoral Training Centre, University of Oxford, Oxford University, United Kingdom, [2]Department of Physics, Oxford University, United Kingdom, [3]Richard D. Berlin Center for Cell Analysis and Modeling, University of Connecticut Health Center, CT, USA, [4]Department of Pharmacology, Oxford University, United Kingdom, [5]Cardiac Biophysics and Systems Biology, Imperial College, United Kingdom.

Flash photolysis of caged-compounds (i.e. photoactivatable compounds) allows one to interact with tissue by controlling the release of bioactive components, both spatially and temporally. The use of ultraviolet light-emitting-diodes (UV LEDs) for uncaging such compounds in cells has recently generated excitement, since LEDs offer high-speed control and are low cost.

Traditional methods for simultaneous imaging of intracellular calcium transients (CaT) and membrane voltage (V_m) are technically challenging and require the use of two cameras. We developed a single-camera (Cascade 128+; Photometrics) approach that images rhod-2 (CaT) and the new near-infrared di-4-ANBDQPQ (V_m), without cross-talk. The technique relies on the isosbestic-point of di-4-ANBDQPQ, a custom-built multi-band optical filter (Chroma Technology) and visible LEDs (Luminus Devices). As the excitation wavelengths of these dyes are significantly longer than those needed to photoactivate caged-compounds, we combined them with a UV-sensitive calcium buffer (NP-EGTA; Invitrogen) to locally modulate free calcium in Langendorff-perfused rat hearts (N=3, Wistar-rat female).

We demonstrate local uncaging of calcium by photo-inhibition of NP-EGTA with a powerful 365nm UV LED (Nichia), while simultaneously imaging CaT and V_m (128x128 pixels; 511 frames-per-second). CaT (shape and kinetics) were dramatically altered, in keeping with reported effects of EGTA-AM in whole-heart studies. The kinetics of CaT recovery changed from biphasic (in the presence of functional EGTA) to monophasic (after photo-inhibition of the buffer), and CaT duration increased.

In summary: Caged-compounds can be used in multi-cellular preparations like the whole-heart to investigate the effects of spatiotemporal perturbations of bioactive compound concentrations, such as calcium buffers, and this approach can be combined with simultaneous measurement of CaT and V_m.

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3410-Pos Board B271

Ca^{2+}/Calmodulin-Dependent Protein Kinase II (CaMKII) Activity Modulates Sinoatrial Nodal Pacemaker Cell Energetics

Yael Yaniv, Harold A. Spurgeon, Bruce D. Ziman, Edward G. Lakatta.
NIA/NIH, Baltimore, MD, USA.

In rabbit sinoatrial node cells (SANC), high basal (i.e., without β-adrenergic receptor stimulation) Ca^{2+} activated adenylate cyclase (AC) via cAMP/PKA-CaMKII-dependent protein phosphorylation guarantees the occurrence of rhythmic intracellular Ca^{2+} release from sarcoplasmic-reticulum which drives spontaneous action potentials (APs). This high-throughput signaling consumes ATP. We have previously demonstrated that basal AC-cAMP/PKA signaling directly, and Ca^{2+} indirectly, not only consume ATP, but also regulate mitochondrial ATP production. A role for CaMKII in regulation of SANC ATP supply has not been explored. Based upon its involvement in the aforementioned phosphorylation signaling cascade we tested the hypotheses that, basal CaMKII activity not only regulates ATP consumption, but also ATP production. We superfused single, isolated rabbit SANC or SANC suspensions with CaMKII inhibitors (KN-93 *or* autocamtide-2 Related Inhibitory Peptide (AIP)) and measured cytosolic Ca^{2+}, cAMP, energetic indices and spontaneous AP firing rate. A partial reduction in basal CaMKII activity by *KN-93 (0.5 μmol/L) or AIP (2 μmol/L)* markedly slowed the kinetics of intracellular Ca^{2+} cycling and decreased the spontaneous AP firing rate. Further suppression of CaMKII activity (KN-93 3 *μmol/L; AIP* 10 *μmol/L*) to a level that eliminates all spontaneous APs, decreased the cAMP level and reduced O_2 consumption and flavoprotein fluorescence. **ATP was depleted,even though the ATP demand decreased**. The structural inactive analog of KN-93, KN-92, neither decreased ATP demand (no effect on spontaneous AP firing rate) nor ATP turnover. Therefore,CaMKII signaling is required not only to drive normal automaticity in rabbit SANC, but is also tightly linked to SANC bioenergetics. Future studies are required to determine whether this link between CaMKII signaling and mitochondrial energetics occurs indirectly, via changes in Ca^{2+} release that affects activation of Ca^{2+}-AC-cAMP/PKA signaling, or directly, via phosphorylation of mitochondrial proteins.

3411-Pos Board B272

An Inherent Ability of Ventricular Myocytes to Self-Organize Spontaneous RyRs Activation that Generate Synchronized Local Ca^{2+} Releases is Physiologically Suppressed by Constitutive Phosphodiesterase and Protein Phosphatase Activity

Syevda Sirenko, Tatiana M. Vinogradova, Victor Maltsev,
Edward G. Lakatta.
NIA/NIH, Baltimore, MD, USA.

Stochastic, spontaneous, local RyRs activation underlies basal "Ca^{2+} sparks" in ventricular myocytes (VM). We have previously shown that addition of exogenous cAMP to saponin-permeabilized rabbit VM synchronizes stochastic Ca^{2+} sparks into rhythmic local Ca^{2+} releases. We hypothesized that in the basal state VM have an intrinsic ability to self-organize spontaneous RyRs activation and synchronize Ca^{2+} releases but this self-organization is suppressed by endogenous phosphodiesterase (PDE) and protein phosphatase (PP) activity, permitting only the rare and stochastic occurrence of Ca^{2+} sparks. We found that